

T 21:18:20 ON 16 DEC 1999)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 21:18:37 ON 16 DEC 1999

L1 1745 S PRIMORDIAL GERM CELLS
L2 209 S L1 AND (AVIAN OR CHICKEN)
L3 2 S L2 AND (TURKEY)
L4 0 S L2 AND (GALLINACEA)
L5 0 S L1 AND GALLINACEA
L6 67 S GALLINACEA
L7 59 DUPLICATE REMOVE L6 (8 DUPLICATES REMOVED)
L8 0 S L7 AND (EMBRYONIC (3W) CELLS)
L9 2 S L7 AND CELLS
L10 87 S L1 AND ((LEUKEMIA INHIBITORY FACTOR) OR (LIF))
L11 36 S L10 AND ((BASIC FIBROBLAST GROWTH FACTOR) OR (BFGF))
L12 25 S L11 AND ((STEM CELL FACTOR) OR (SCF))
L13 1 S L12 AND ((INSULIN-LIKE GROWTH FACTOR) OR (IGF))
L14 15 DUPLICATE REMOVE L12 (10 DUPLICATES REMOVED)
L15 4 S L2 AND L10
L16 2 S L2 AND L11
L17 2 S L2 AND L12
L18 2 S L2 AND L12
L19 9 S L1 AND (CULTURE (10W) DAYS)
L20 0 S (PONCE DE LEON) AND L1
L21 0 S ROBL/AU
L22 156 S STICE?/AU
L23 2 S L22 AND L1
L24 1 S (PONCE DE LEON/AU)
L25 0 S F. ABEL PONCE DE LEON/AU
L26 59 S ROBL, JAMES M/AU
L27 1 S L26 AND L1
L28 21 S (PONCE DE LEON, F. ABEL/AU)
L29 1 S L28 AND L1
L30 28 S JERRY, D. JOSEPH/AU
L31 1 S L30 AND L1

L19 ANSWER 7 OF 9 CAPLUS COPYRIGHT 1999 ACS
 AB **Primordial germ cells** are extd. from post
 blastocyst embryos of an ungulate such as extg. **primordial**
germ cells from the gonadal ridges of 25-day porcine
 embryos or 34-40-day bovine embryos. The **primordial**
germ cells are cultured in long-term **culture**
 (>30 **days**) resulting in cells which resemble embryonic stem
 cells in morphol. and with respect to maintaining pluripotency. The
 cells
 obtained can. . .

=> d 7 bib

L19 ANSWER 7 OF 9 CAPLUS COPYRIGHT 1999 ACS
 AN 1997:528715 CAPLUS
 DN 127:118261
 TI Ungulate embryonic stem-like cells: making and using the cells to produce
 a transgenic ungulate
 IN Anderson, Gary B.; Shim, Hosup
 PA Regents of the University of California, USA
 SO PCT Int. Appl., 36 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9725413	A1	19970717	WO 1996-IB16	19960109
	W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AZ, BY, KZ, RU, TJ,			
TM		RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG		
	AU 9643536	A1	19970801	AU 1996-43536	19960109
	WO 9725412	A1	19970717	WO 1997-US41	19970108
	W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
	CA 2242666	AA	19970717	CA 1997-2242666	19970108
	AU 9715706	A1	19970801	AU 1997-15706	19970108
	EP 882127	A1	19981209	EP 1997-901907	19970108
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
	CN 1212009	A	19990324	CN 1997-192440	19970108
PRAI	WO 1996-IB16		19960109		
	US 1996-717155		19960920		
	WO 1997-US41		19970108		

L14 ANSWER 3 OF 15 BIOSIS COPYRIGHT 1999 BIOSIS

AB In recent years, much attention has been paid to the manipulation of **primordial germ cells** (PGCs) to produce transgenic chicken. For this purpose, the in vitro proliferation of PGCs would make gene targeting possible, promoting the efficiency of transgenes. However, few researches have been carried out on this topic. In the present study, we tried to culture chicken PGCs outside the body. The PGCs were isolated from circulating blood of chicken embryos by the method of Ficoll density centrifugation, and labeled with PKH26 red fluorescent cell linker. The cells were refined by selection with a fine glass pipette and a micromanipulator in order to avoid the contamination of PGCs with red cells. The refined cells were then cultured for a given period. The PGCs proliferated when they were cultured on feeder cells derived from the germinal ridges of chicken embryos at stage 27. During a 5 day culture period, the highest rate of increased PGCs was about 51%

for

one of the two test groups. Although some kinds of growth factors were supplemented in the culture media, no synergistic effect of chicken **stem cell factor** (chSCF) with murine **leukemia inhibitory factor** (LIF) and human **basic fibroblast growth factor** (bFGF) on the proliferation of PGCs was found. This result implies that the LIF and bFGF influencing the proliferation of PGCs may not be conserved between mammals and birds.

=> d 3 bib

L14 ANSWER 3 OF 15 BIOSIS COPYRIGHT 1999 BIOSIS

AN 1999:409207 BIOSIS

DN PREV199900409207

TI Survival and proliferation of refined chicken circulating **primordial germ cells** cultured in vitro.

AU Yang, Guoqing; Fujihara, Noboru (1)

CS (1) Department of Animal Sciences, College of Agriculture, Kyushu University, Hakozaki, Fukuoka, 812-8581 Japan

SO Journal of Reproduction and Development, (April, 1999) Vol. 45, No. 2,

PP.

177-181.

ISSN: 0916-8818.

DT Article

LA English

SL English

Handwritten signature

3

L15 ANSWER 1 OF 4 MEDLINE

AB Fluorescent reagent-labelled PGCs isolated from the blood of 2-day-old chick embryos were cultured on stroma cells derived from 5-day-old germinal ridge in Medium 199 supplemented with 10% FBS, human IGF-1, bovine FGF-b, and murine **LIF**. In 7 experiments, the number of MCs increased by an average of 4.8 fold in 4 days. Intrinsic PGCs in the 5-day embryonic germinal ridge were observed loosely attached to the stroma cells, and they also increased 3.8 fold during culture for 4 days. These results indicate the possibility of applying this culture method to the production of transgenic **chickens**.

=> d 1 bib

L15 ANSWER 1 OF 4 MEDLINE

AN 95261329 MEDLINE

DN 95261329

TI Proliferation of chick **primordial germ cells** cultured on stroma cells from the germinal ridge.

AU Chang I K; Tajima A; Chikamune T; Ohno T

CS RIKEN Cell Bank, Institute of Physical and Chemical Research (RIKEN), Ibaraki, Japan..

SO CELL BIOLOGY INTERNATIONAL, (1995 Feb) 19 (2) 143-9.
Journal code: BPN. ISSN: 1065-6995.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199508

+ Pcsce

Turkey primordial germ cells

transferred by intravascular injection to previously sterilized chick embryos can undergo complete maturation inside the host's gonads and can give rise to gametes which are more or less suitable for fertilization. The resulting spermatozoa fertilized hen eggs at a higher frequency than normal **turkey** spermatozoa, but without allowing a longer or a more normal development. It was impossible to fertilize **turkey** eggs with them. The resulting eggs sometimes had an abnormal-looking yolk and were laid during the first 7 mo. only. Brought in contact with **chicken** spermatozoa, they were fertilized (or perhaps merely activated), but they never gave rise to embryos. Fertilized by **turkey** spermatozoa, they developed into embryos, sometimes abnormal, which in the best case reached the 15th day of incubation (stage 38 HH [Hamilton-Hamburger]). Some praepennae of the latter embryo showed a red-brown pigment which cannot be determined by the genotype of the zygote (a white **turkey**'s) and which resembled the phenotype of the foster mother (a red-brown hen). After intraspecific transfer of **primordial germ cells**, maturation of Rhode Island Red oocytes inside a Wyandotte White ovary (in 2 hens) and vice versa (in 1 hen) was achieved. Laying was also possible but often at a lower frequency than normal. When a Wyandotte White hen bearing Rhode Island Red oocytes was mated with a normal Rhode Island cock, the down of their offspring looked brighter than Rhode Island Red **chicken**'s in one case, but it was subsequently replaced by red-brown feathers according to the genotype. When a Rhode Island Red hen bearing Wyandotte White oocytes was mated with a normal Wyandotte White cock, the down of their offspring was never in agreement with the genotype. It always showed a black pigment over more or less large areas and, in one case, a red-brown pigment, both of which were present in the foster mother. The origin and the mechanism of such a transfer of pigments are not understood. It might represent merely a temporary effect acting upon the down of the first generation. As far as the comb is concerned, it was always in agreement with the original genotype.

=> d 1 bib

L3 ANSWER 1 OF 2 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1977:179843 BIOSIS
DN BA64:2207
TI REPRODUCTIVE CAPACITY AND OFFSPRING OF **CHICKENS** SUBMITTED TO A
TRANSFER OF **PRIMORDIAL GERM CELLS** DURING
EMBRYONIC LIFE.
AU REYNAUD G
SO WILHELM ROUX'S ARCH DEV BIOL, (1976) 179 (2), 85-110.
CODEN: WRABDT. ISSN: 0340-0794.
FS BA; OLD
LA Unavailable

L14 ANSWER 14 OF 15 MEDLINE

DUPLICATE 7

AB Recent studies have shown that **stem cell factor, leukemia inhibitory factor, and basic fibroblast growth factor** increase proliferation and survival of the mouse **primordial germ cells** (PGCs) in culture. We now show that addition of tumor necrosis factor-alpha (TNF-alpha) to culture medium stimulates proliferation of PGCs without transforming them into embryonic stem cells and that its effect is specific for the PGCs at younger stages before and during their migration to gonads. A previously reported finding that TNF-alpha is expressed at these stages in mouse embryos suggests possible involvement of TNF-alpha in the proliferative regulation of the PGCs in the embryo.

AN 94123873 MEDLINE

DN 94123873

TI Tumor necrosis factor-alpha (TNF-alpha) stimulates proliferation of mouse **primordial germ cells** in culture.

AU Kawase E; Yamamoto H; Hashimoto K; Nakatsuji N

CS Mammalian Development Laboratory, National Institute of Genetics, Mishima, Japan..

SO DEVELOPMENTAL BIOLOGY, (1994 Jan) 161 (1) 91-5.
Journal code: E7T. ISSN: 0012-1606.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 199405

L8 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS

AN 1994:212063 CAPLUS

DN 120:212063

TI **Insulin-like growth factor II**

(IGF-II) and leukemia-inhibiting factor (**LIF**) for culturing animal **embryonic stem cells**

IN Takahashi, Akio; Takahashi, Yumi; Matsumoto, Kazuya; Myata, Kenji

PA Enu Teii Saiensu Kk, Japan

SO Jpn. Kokai Tokkyo Koho, 8 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	---	-----	-----	-----
PI	JP 06038742	A2	19940215	JP 1992-83866	19920406

L8 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1999 ACS

AN 1994:161798 CAPLUS

DN 120:161798

TI **Insulin-like growth factor II**

(IGF-II) for culture of animal undifferentiated embryonic cells

IN Takahashi, Yumi; Takahashi, Akio; Matsumoto, Kazuya; Myata, Kenji

PA Enu Teii Saiensu Kk, Japan

SO Jpn. Kokai Tokkyo Koho, 9 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	---	-----	-----	-----
PI	JP 05304951	A2	19931119	JP 1992-83867	19920406

=> d 2 ab

L8 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS

AB IGF-II and **LIF** are used for culturing, establishing or maintaining an mammalian, avian or fish embryonic stem cell line.

=> d 3 ab

L8 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1999 ACS

AB IGF-II with/without addnl. leukemia inhibition factor (**LIF**) is useful for culturing undifferentiated animal cells such as embryonal carcinoma cell (EC cell) or embryonic stem cell (ES cell), and establishment of the cells into cell lines. IGF-II inhibits the differentiation of the animal undifferentiated cells and promotes the growth of the same. Establishment of ES cell lines from **embryonic stem cells** of Wistar and ACI rats was shown.

L19 ANSWER 9 OF 9 CAPLUS COPYRIGHT 1999 ACS

AB Recent studies have shown that stem cell factor (SCF), leukemia inhibitory

factor (LIF), basic fibroblast growth factor (bFGF) and the enhancement of

cAMP levels increase proliferation and survival of mouse

primordial germ cells (PGC) in vitro. Even

after the addn. of these factors, however, it is still not possible to

obtain proliferation of PGC at a rapid rate similar to that in vivo,

suggesting the presence of other growth factor(s) in vivo. We previously

reported that tumor necrosis factor- α . stimulates proliferation of

PGC at earlier migration stages. We now show that the use of SI/SI4-m220

feeder cells and the addn. of a medium conditioned with Buffalo rat liver

cells and forskolin to the **culture** medium stimulate PGC obtained

from 8.5 **days** post coitum embryos to proliferate in culture at a

rate comparable to that in vivo. Under such conditions, proliferation of

PGC continued several days past the timing of growth arrest in vivo;

however, it did stop afterward. Such proliferating PGC continued to

express c-kit and Oct-3 proteins. The characteristics of the culture

medium and the requirement of feeder cells were different from those for

embryonic stem (ES) cells, suggesting that these rapidly proliferated PGC

are not transformed into ES-like EG cells.

AN 1996:487602 CAPLUS

DN 125:159418

TI A combination of Buffalo rat liver cell-conditioned medium, forskolin and membrane-bound stem cell factor stimulates rapid proliferation of mouse

primordial germ cells in vitro similar to that

in vivo

AU Kawase, Eihachiro; Shirayoshi, Yasuaki; Hashimoto, Koichiro; Nakatsuji, Norio

CS Mammalian Development Lab., Natl. Inst. Genetics, Mishima, 411, Japan

SO Dev., Growth Differ. (1996), 38(3), 315-322

CODEN: DG DFA5; ISSN: 0012-1592

DT Journal

LA English

L14 ANSWER 13 OF 15 MEDLINE

AB Information obtained mainly from in vitro culture studies and genetic analysis of mouse mutants White spotting and Steel indicate a pivotal role

of growth factors in the development of mouse **primordial germ cells** (PGCs). While **stem cell factor** (**SCF**) and TGF beta 1 seem to have a role in PGC migration (as an adhesion factor and a chemoattractant, respectively), the

former is certainly required for PGC survival in vitro and probably in vivo as well. Recent findings suggest that the mechanism by which **SCF** supports PGC survival is by preventing PGC apoptosis. A similar action appears to be exerted by **leukemia inhibitory factor** (**LIF**), a further growth factor influencing PGC growth in culture. PGC proliferation seems to be mainly induced by cAMP dependent mechanisms, but further investigations are needed to clarify the interrelationships among the different

molecular

pathways activated by **SCF**, **LIF**, cAMP and other putative PGC growth factors (i.e. **bFGF**). Stimulation of long-term proliferation of PGCs, leading to derivation of ES-like cells (embryonal germ cells) obtained by using a combination of growth factors

(**bFGF**, **SCF** and **LIF**), opens new intriguing perspectives for such studies and transgenic technology.

AN 95002789 MEDLINE

DN 95002789

TI Growth factors in mouse primordial germ cell migration and proliferation.

AU De Felici M; Pesce M

CS Department of Public Health and Cell Biology, University of Rome Tor Vergata, Italy..

SO PROGRESS IN GROWTH FACTOR RESEARCH, (1994) 5 (2) 135-43. Ref: 35
Journal code: A6S. ISSN: 0955-2235.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LA English

FS Priority Journals

EM 199501

L19 ANSWER 5 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS

AB **PRIMORDIAL germ cells** (PGCs) are first identifiable as a population of about eight alkaline phosphatase-positive cells in the 70 days postcoitum mouse embryo-1. During the next 6 days of development they proliferate to give rise to the 25,000 cells that will establish the meiotic population-2. Steel factor is required for PGC survival both in vivo-3 and in vitro-4,5 and together with leukaemia inhibitory factor stimulates PGC proliferation in vitro-6. In feeder-dependent **culture**, PGCs will proliferate for up to 7 **days**, but their numbers eventually decline and their proliferative capacity is only a fraction of that seen in vivo-6,7. Here we report a further factor that stimulates PGC proliferation in vitro, basic fibroblast growth factor (bFGF). Furthermore, bFGF, in the presence of steel factor and leukaemia inhibitory factor, stimulates long-term proliferation of PGCs, leading to the derivation of large colonies of cells. These embryonic germ cells resemble embryonic stem cells, pluripotent cells derived from preimplantation embryos, or feeder-dependent embryonal carcinoma cells, pluripotent stem cells of PGC-derived tumours (teratomas and teratocarcinomas)-8. To our knowledge, these results provide the first system for long-term culture of PGCs.

=> d 119 bib

L19 ANSWER 1 OF 9 MEDLINE

AN 95261329 MEDLINE

DN 95261329

TI Proliferation of chick **primordial germ cells** cultured on stroma cells from the germinal ridge.

AU Chang I K; Tajima A; Chikamune T; Ohno T

CS RIKEN Cell Bank, Institute of Physical and Chemical Research (RIKEN), Ibaraki, Japan..

SO CELL BIOLOGY INTERNATIONAL, (1995 Feb) 19 (2) 143-9.

Journal code: BPN. ISSN: 1065-6995.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199508

L4 ANSWER 1 OF 2 MEDLINE
 AN 97176436 MEDLINE
 DN 97176436
 TI Origin of **primordial germ cells** in the
 prestreak chick embryo.
 AU Karagenc L; Cinnamon Y; Ginsburg M; Petitte J N
 CS Department of Poultry Science, North Carolina State University, Raleigh,
 NC 27695, USA.
 NC NO1-HD-2-3144 (NICHD)
 SO DEVELOPMENTAL GENETICS, (1996) 19 (4) 290-301.
 Journal code: DEG. ISSN: 0192-253X.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199705
 EW 19970503

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2000 ACS
 AN 2000:87092 CAPLUS
 DN 132:149249
 TI Soluble factors and the emergence of chick **primordial
 germ cells** in vitro
 AU Karagenc, L.; Petitte, J. N.
 CS Department of Poultry Science, North Carolina State University, Raleigh,
 NC, 27695-7608, USA
 SO Poult. Sci. (2000), 79(1), 80-85
 CODEN: POSCAL; ISSN: 0032-5791
 PB Poultry Science Association, Inc.
 DT Journal
 LA English
 RE.CNT 26
 RE
 (1) Bartunek, P; Cytokine 1996, V8, P14 CAPLUS
 (3) Davis, S; Science (Washington DC) 1993, V260, P1805 CAPLUS
 (4) Dolci, S; Mol Reprod Dev 1993, V35, P134 CAPLUS
 (5) Dolci, S; Nature (Lond) 1991, V352, P809 CAPLUS
 (6) Donovan, P; Curr Top Dev Biol 1994, V29, P189 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d 1 ab

L4 ANSWER 1 OF 2 MEDLINE
 AB The temporal and spatial pattern of segregation of the **avian**
 germline from the formation of the area pellucida to the beginning of
 primitive streak formation (stages VII-XIV, EG&K) was investigated using
 the **culture** of whole embryos and central and peripheral embryo
 fragments on vitelline membranes at stages VII-IX, immunohistological
 analysis of whole mount embryos and sections with monoclonal antibodies
 MC-480 against stage-specific embryonic antigen-1 (SSEA-1) and EMA-1, and
 with the **culture** of dispersed blastoderms at stages IX-XIV with
 and **without** on STO **feeder layer**. Whole
 embryos at intrauterine stages developed up to the formation of the
 primitive streak despite the **absence** of area pellucida
 expansion. **Primordial germ cells** (PGCs)

appeared in the **cultures** of whole embryos and only in central fragments containing a partially formed area pellucida at stages VII-IX. When individual stage IX-XIV embryos were dispersed and cultured **without a feeder layer**, 25-45 PGCs/embryo were detected only with stage X-XIV, but not with stage IX blastoderms. However, the **culture** of dispersed cells from the area pellucida of stages IX-XIII on STO **feeder layers** yielded about 150 PGCs/embryo. The carbohydrate epitopes recognized by anti-SSEA-1 and EMA-1 first appeared at stage X on cells in association with polyngressing cells on the ventral surface of the epiblast and later on the dorsal surface of the hypoblast. The SSEA-1-positive hypoblast cells gave rise to chicken PGCs when cultured on a **feeder layer** of quail blastodermal cells. From these observations, we propose that the segregation and development of **avian** germline is a gradual, epigenetic process associated with the translocation of SSEA-1/EMA-1-positive cells from the ventral surface of the area pellucida at stage X to the dorsal side of the hypoblast at stages XI-XIV.

=> d 2 ab

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2000 ACS

AB Previous observations obtained from a **culture** of blastodermal cells on a mouse fibroblast **feeder layer** (STO) suggested that STO cells provide a factor or factors that facilitate development of **avian primordial germ cells** (PGC) from dispersed embryo cells. The purpose of the current study was to test the hypothesis that sol. factors produced by

STO cells are responsible, at least in part, in supporting the development of PGC in **culture** and to examine the effect of stem cell factor (SCF), ciliary neurotrophic factor (CNTF), and basic fibroblast growth factor (bFGF) in the development of PGC in **culture**. Blastodermal cells on gelatin-coated plastic or on **feeder layers** of CV-1 cells yielded a small **no.** of PGC. When blastodermal cells were cultured on STO cells, a marked increase in PGC was obsd. The addn. of STO cell-conditioned medium (STO-CM) to blastodermal cells cultured on gelatin-coated plastic and on **feeder layers** of CV-1 cells resulted in a significant increase in the **no.** of PGC, indicating that sol. factors produced by STO cells can enhance the development of chicken PGC in **culture**. Supplementation of blastodermal cells with SCF (100 ng/mL) or CNTF (2 ng/mL) or with CNTF and SCF together resulted in a significant increase in the **no.** of PGC after 48 h of **culture** on **feeder layers** of CV-1 cells. However, addn. of bFGF (100 ng/mL) did not increase PGC. We concluded from these observations that the **culture** of blastodermal cells on **feeder layers** of STO and CV-1 cells can be used as a useful biol. system in examg. the regulatory factors that govern the ontogeny of the germ cell lineage in the **avian** embryo.

L5 ANSWER 6 OF 9 MEDLINE

AB Two agents known to enhance the level of intracellular cAMP (dibutyryl cAMP and forskolin) markedly increase the number of 8.5, 10.5, and 11.5 days postcoitum (dpc) mouse **primordial germ cells** (PGCs) cultured on TM4 cell **feeder layers**. Forskolin (FRSK) caused a significant increase of PGC number also in monodispersed cell suspensions obtained from PGC-containing tissues of the three embryonic ages studied and in purified 11.5 dpc PGCs cultured **without feeder layers**. The addition to the **culture** medium of adenosine-3',5'-cyclic monophosphorothioate RP isomer (Rp-cAMPS, a competitive antagonist for cAMP-dependent protein kinases), significantly reduced the effects of FRSK. Last, FRSK stimulated PGC proliferation, as assessed by 5-bromo-2'-deoxyuridine incorporation. We conclude that cAMP-dependent mechanisms play a crucial role in the control of mitotic proliferation of mouse PGCs in **culture**.

=> d 6 bib

L5 ANSWER 6 OF 9 MEDLINE

AN 93246070 MEDLINE

DN 93246070

TI Proliferation of mouse **primordial germ cells**
in vitro: a key role for cAMP.

AU De Felici M; Dolci S; Pesce M

CS Department of Public Health and Cell Biology, II University of Rome, Italy.

SO DEVELOPMENTAL BIOLOGY, (1993 May) 157 (1) 277-80.
Journal code: E7T. ISSN: 0012-1606.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 199308

L5 ANSWER 5 OF 9 MEDLINE

AB In the present paper we investigated the effects of stem cell factor/mastocyte growth factor (SCF/MGF), leukemia inhibitory factor/differentiating inhibitory activity (LIF/DIA) (two growth factors known to affect primordial germ cell growth in vitro) and forskolin

(FRSK)

(an activator of adenylate cyclase in many cell types) alone or in combination on the survival and proliferation of **primordial germ cells** (PGCs) obtained from 8.5, 10.5, and 11.5 days post coitum (dpc) mouse embryos and cultured **without** pre-formed cell **feeder layers**. The results showed that both at 1 and 3 days of **culture** the addition of 100 ng/ml SCF, 20 microm FRSK, or in some instances 20 ng/ml LIF alone caused a significant increase of PGC number as compared with controls. The highest effects

were

obtained when SCF and/or LIF were used together with FRSK. Moreover, we found that FRSK elevated cAMP levels in purified 11.5 dpc PGCs and that this compound, but not SCF and LIF, stimulated PGC proliferation, as assessed by 5-bromo-2'-deoxyuridin (BrdU) incorporation. These results suggest a mechanism of combined action of cAMP with SCF and/or LIF in the control of proliferation of mouse PGCs in vitro.

=> d 5 bib

L5 ANSWER 5 OF 9 MEDLINE

AN 93305298 MEDLINE

DN 93305298

TI Combined action of stem cell factor, leukemia inhibitory factor, and cAMP on in vitro proliferation of mouse **primordial germ cells**.

AU Dolci S; Pesce M; De Felici M

CS Department of Public Health and Cell Biology, University of Rome Tor Vergata, Italy..

SO MOLECULAR REPRODUCTION AND DEVELOPMENT, (1993 Jun) 35 (2) 134-9.
Journal code: AN7. ISSN: 1040-452X.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199310

L5 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2000 BIOSIS

AB The in vitro **culture** conditions allowing survival and initial proliferation of murine **primordial germ cells** from 10.5 days post coitum embryos, which include the use of a murine embryonal fibroblast (STO) feeder, were applied to 21 human seminomas, composed of tumour cells which are considered as the malignant counterparts of human **primordial germ cells**. Cells from 18 seminomas attached poorly to STO, and only a few survived through day 10. In contrast, three seminomas showed a higher degree of attachment. Two of them showed initial proliferation and enhanced survival: 30 days for tumour SE1 and 25 days for tumour SE3. Tumour SE1 was more extensively studied, using the **culture** conditions allowing the derivation of pluripotent feeder, stem cell factor, leukaemia inhibitory factor and basic fibroblast growth factor. The presence of stem cell factor was necessary and sufficient for colonies of turnout cells to form during the first 3 days of **culture**. While the cell number decreased after day 3 in medium **without** fetal calf serum, it increased until day 9 in medium containing fetal calf serum. **No** reprogramming of SE1 cells to pluripotent stem cells was observed. Our data indicate that seminomas form a tumour population with a heterogeneous in vitro behaviour not equivalent to that of 8.5 - 10.5 days post coitum murine **primordial germ cells**.

=> d 8 bib

L5 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2000 BIOSIS

AN 1995:111219 BIOSIS

DN PREV199598125519

TI Heterogeneity in the in vitro survival and proliferation of human seminoma cells.

AU Olie, R. A. (1); Looijenga, L. H. J.; Dekker, M. C.; De Jong, F. H.; Van Dissel-Emiliani, F. M. F.; De Rooij, D. G.; Van Der Holt, B.; Oosterhuis, J. W.

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DT Article

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L5 ANSWER 7 OF 9 MEDLINE
 AB Mutations at the steel (sl) and dominant white spotting (W) loci in the mouse affect **primordial germ cells** (PGC), melanoblasts and haemopoietic stem cells. The W gene encodes a cell-surface receptor of the tyrosine kinase family, the proto-oncogene c-kit. In situ analysis has shown c-kit messenger RNA expression in PGC in the early genital ridges. The Sl gene encodes the ligand for this receptor, a peptide growth factor, called here stem cell factor (SCF). SCF mRNA is expressed in many regions of the early mouse embryo, including the areas of migration of these cell types. It is important now to identify the role of the Sl-W interaction in the development of these migratory embryonic stem cell populations. Using an in vitro assay system, we show that SCF increases both the overall numbers and colony sizes of migratory PGC isolated from wild-type mouse embryos, and cultured on irradiated **feeder layers** of STO cells (a mouse embryonic fibroblast line). In the **absence** of feeder cells, SCF causes a large increase in the initial survival and apparent motility of PGC in **culture**. But labelling with bromodeoxyuridine shows that SCF is not, by itself, a mitogen for PGC. SCF does not exert a chemotropic effect on PGC in in vitro assays. These results suggest that SCF in vivo is an essential requirement for PGC survival. This demonstrates the control of the early germ-line population by a specific trophic factor.

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L5 ANSWER 7 OF 9 MEDLINE
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 TI Effects of the steel gene product on mouse **primordial germ cells** in **culture**.
 AU Godin I; Deed R; Cooke J; Zsebo K; Dexter M; Wylie C C
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